



QUANTIFICATION AFTER TRANSIT IN HUMAN DIGESTIVE TRACT OF LACTOBACILLUS R0011 AND LACTOBACILLUS R0052 CONSUMED IN A FOOD SUPPLEMENT

O. Firmesse, J. P. Furet, A. Mogenet, J. L. Bresson and G. Corthier

National Institute of Agronomic Research. Research, Unit on Ecology and Physiology of Digestive System, Jouy-en-Josas, France

Introduction

Previous studies have shown that probiotics improve intestinal metabolism, modulate immunity, and prevent intestinal pathologies like diarrhoea. These effects are dependent on the food matrix and the included probiotic strains. The aim of this present study was to evaluate the ability of *Lactobacillus* R0011 and *Lactobacillus* R0052, ingested in food supplement, to survive the passage through the human digestive tract. To assess this point, selective enumeration and real time PCR were used to quantify the amount of *Lactobacillus* R0011 and *Lactobacillus* R0052 in faecal samples.

Clinical study design

The clinical study performed was an open human protocol. Fourteen healthy volunteers (7 males, 7 females) were recruited into the study at Necker Hospital, Paris, France. They had no antibiotic treatment for 3 months prior to the study, no acute or chronic diseases and no gastrointestinal problems. During the investigation period, the only restriction with regards to diet was the exclusion of fermented products. The total experiment time was 7 weeks divided into 3 different parts: a 3-week exclusion period of fermented products, followed by a 2-week consumption period of capsules and a 2-week wash-out period. During the consumption period, the subjects consumed daily 4 capsules each containing 2×10^9 *Lactobacillus* R0011 and 1×10^8 *Lactobacillus* R0052. Seven faecal samples were collected: 2 during the exclusion period (between day-7 and day-3 before the ingestion period), 3 during the consumption period (between day+8 and day+12) and 2 faecal samples during the wash-out period: the first one between day+15 and day+17 and the last one at the end of the second week (between day+22 and day+24).

Lactobacillus R0011 and *Lactobacillus* R0052 survival in faeces after consumption of food supplement: semi-selective bacterial enumeration

Survival of *Lactobacillus* R0011 and *Lactobacillus* R0052 was estimated for all volunteers and for each faecal sample. Faecal samples were homogenized, serially diluted and each dilution was evenly spread on plates of MRS-agar containing $50 \mu\text{g}\cdot\text{mL}^{-1}$ vancomycin for selective recovery of *Lactobacillus* R0011 and $20 \mu\text{g}\cdot\text{mL}^{-1}$ ciprofloxacin and $10 \mu\text{g}\cdot\text{mL}^{-1}$ tetracyclin for selective recovery of *Lactobacillus* R0052. The plates were incubated in anaerobic for 2 days at 42°C , after which R011 and R0052 colony-forming units (CFU) were counted. Results were expressed as the logarithm (log) of number of $\text{CFU}\cdot\text{g}^{-1}$ of stool. The detection limit was $2.0 \log \text{CFU}\cdot\text{g}^{-1}$ of stool. The media were not fully selective and for some volunteers the autochthonous bacteria interfered with the enumerations.

At the end of the exclusion period, no *Lactobacillus* R0011 or *Lactobacillus* R0052 colony was recovered on selective plates, indicating the probable absence of the two strains in the faecal microbiota of all subjects before capsule consumption. During the period of intake, viable *Lactobacillus* R0011 cells were counted from all the volunteers at levels between 2.3 and $5.9 \log \text{CFU}\cdot\text{g}^{-1}$ of stool, with a median value of $3.8 \log \text{CFU}\cdot\text{g}^{-1}$ of stool. For *Lactobacillus* R0052, viable cells were recovered from 10 out of 14 volunteers at levels between 2.3 and $4.5 \log \text{CFU}\cdot\text{g}^{-1}$ of stool, with a median value of $2.0 \log \text{CFU}\cdot\text{g}^{-1}$ of stool. No *Lactobacillus* R0052 was found in the majority of faecal samples (26 out of 42).

One week after the end of capsule consumption, *Lactobacillus* R0011 cells were still detectable in 5 volunteers out of 14 at levels between 2.3 and $4.2 \log \text{CFU}\cdot\text{g}^{-1}$ of stool, whereas no *Lactobacillus* R0052 cells was found. At the end of the second week of the post ingestion period, no *Lactobacillus* R0011 or *Lactobacillus* R0052 cells were recovered on plates.

These data suggested that *Lactobacillus* R0011 had a better survival than *Lactobacillus* R0052. But it has to be considered that the latter was 10 times less concentrated in the capsules. The *Lactobacillus* R0011 survival seemed to be small when related to the global consumption but the technique used probably underestimated the real survival since the autochthonous microbiota interfere with the enumeration and the selective antibiotic pressure could have a negative effect on bacteria revival.

***Lactobacillus* R0011 and *Lactobacillus* R0052 survival in faeces after consumption of food supplement: estimation by real time quantitative PCR**

Real time quantitative PCR was carried out on total cellular DNA extracted from 0.2 g of each faecal sample. The 16S rRNA gene fragments of *Lactobacillus* R0011 and *Lactobacillus* R0052 were amplified using two specific primer pairs. To calibrate the method, for each set of primers, a standard curve was made with total genomic DNA obtained from known concentrations of CFU of the two strains. These reference curves allow data expression as a numerical value of bacterial cells per gram of faeces. All the DNA preparations were diluted 1/000 due to the presence of inhibitors in numerous samples, estimated as described by Furet et al. (2004). To determine the detection limit of 16S rDNA amplification by real-time PCR assays in faecal sample, DNAs extracted from known amounts of *Lactobacillus* R0011 and *Lactobacillus* R0052 were added in serial dilutions from 9.0 to 4.0 log cells.g⁻¹ of stool. The detection limit determined in this study was 5.0 log cells.g⁻¹ of stool.

At the end of the exclusion period preceding the capsules consumption, no *Lactobacillus* R0011 or *Lactobacillus* R0052 was found in stools, confirming the absence of the two strains in the faecal samples of all subjects before capsule intake. During the capsules consumption, high levels of *Lactobacillus* R0011 cells were observed in faecal samples from all the volunteers, reaching levels between 6.3 and 8.5 log bacterial cells.g⁻¹ of stool, with a median value of 7.1 log bacterial cells.g⁻¹ of stool. *Lactobacillus* R0052 cells were only detected in stools from one volunteer, reaching maximal levels of 6.1 log bacterial cells.g⁻¹ of stool whereas the detection limit determined in this study was 5.0 log bacterial cells.g⁻¹ of stool. One week after the end of capsule consumption, *Lactobacillus* R0011 cells were detected from 4 volunteers out of 14 at levels between 5.5 and 7.1 log bacterial cells.g⁻¹ of stool, whereas no *Lactobacillus* R0052 cells were detected. At the end of the second week of the post ingestion period, the presence of *Lactobacillus* R0011 cells was still detectable in 2 volunteers at levels between 5.8 and 6.4 log bacterial cells.g⁻¹ of stool.

These results were in some instances confirmation that *Lactobacillus* R0011 survived in faecal samples after transit. The real time quantitative PCR is more sensitive and selective than the direct enumeration which seemed to underestimate the survival. But this fact was not observed for the *Lactobacillus* R0052 strain which is rarely detected by the 2 techniques used here. However, the adhesion properties of this strain might enable him to be active in the ileum. It is a hypothesis to be tested.

References

Furet J.P., Quenee P., Tailliez P., 2004. Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. *Int. J. Food Microbiol.*, 97: 197-207.